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CRISPR/Cas9: Development and Application in *Oriza sativa* Breeding

Aashruti Bhadoria
Amity Institute of Biotechnology, Amity University Madhya Pradesh
Email: aashruti1026@gmail.com

Targeted nucleases for genome editing, such as Zinc finger nucleases (ZFNs) and transcription activator like effector nucleases (TALENS), are important tools in plants for understanding gene functioning and developing useful new traits. The frequently interspersed clustered short palindromic repeats (CRISPR)/Cas system has recently emerged as an alternative approach for successful and scalable genome engineering based on nuclease. To target different genes, only the 20-nt targeting sequence within thesingle-guide RNA (sgRNA) needs to be modified in this method. In this study, the sgRNA scaffold vectors were cloned form target-specific sgRNA oligos. Further it is processed for the isolation of protoplasts, followed by the PEG mediated transformation of protoplast and includes the genome DNA extraction. The process is further continued with the detecting of the mutations in protoplasts and the stable transformation of rice using the biolistic method and the detection and sequencing of indels in transgenic rice plants. The simplicity of the cloning method and the few constraints on possible target sites make the framework of CRISPR/Cas very attractive. In protoplast, the CRISPR/Cas system provides a simple technique for rapid gene targeting within 1-2 weeks, mutated rice plants can be produced within 1317 weeks.

Keywords: CRISPR/Cas9; Oriza sativa; Ggene engineering; Indels; Single-guide RNA